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SPECTRAL SENSITIVITIES OF PHOTORECEPTORS IN THE OCELLI OF THE TARANTULA *APHONOPELMA CHALCODES* (ARANEAE, THERAPHOSIDAE)

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ABSTRACT

Spectral sensitivities of primary and secondary eyes in the Theraphosid spider, *Aphonopelma chalcodes* Chamberlin, were investigated by recording intracellular receptor potentials from single photoreceptors. The responses of all cells were graded depolarizations, monophasic in waveform. All cells showed dual spectral sensitivities, with a primary peak near 500 nm and a secondary peak in the near ultraviolet at 370 nm. The 500 nm peaks were fit well by a Dartnall nomogram.

Spectral sensitivity curves were similar under both dark and light adaptation suggesting the presence of a single photopigment. Intensity-response functions with *white* light showed sensitivity differences between primary and secondary eyes. Secondary eyes had greater sensitivity ranges and smaller slope coefficients showing them to be more sensitive than primary eyes.

INTRODUCTION

Differing spectral sensitivities of visual cells are of considerable interest because they are an essential condition for wavelength discriminations in visual behavior. In several species of spider, multiple and differentiable sensitivities have been reported to both visible and to near-ultraviolet spectral regions: in the wolf spider, *Lycosa* (DeVoe, Small and Zvargulis 1969; DeVoe 1972); in the jumping spider, *Phidippus* (DeVoe 1975), in *Menemerus* (Yamashita and Tateda 1976, 1981), in *Plexippus* (Blest, Hardie, McIntyre and Williams 1981); and in *Argiope* (Yamashita and Tateda 1976, 1978, 1981; Tiedemann, Ventura and Ades 1986).

In this paper, we report spectral sensitivities from the visual cells of ocelli in another spider, the New World Tarantula *Aphonopelma chalcodes* Chamberlin. The arrangement of ocelli consisting of primary and secondary eyes is shown in Figure 1. Spectral sensitivities were measured from single photoreceptor cells in each of the ocelli by means of intracellular recording. Both intensity-response functions and action spectra were derived from the basic recordings. Comparative evaluations were made among the several ocelli; comparisons were also drawn between *Aphonopelma* and other arthropods.

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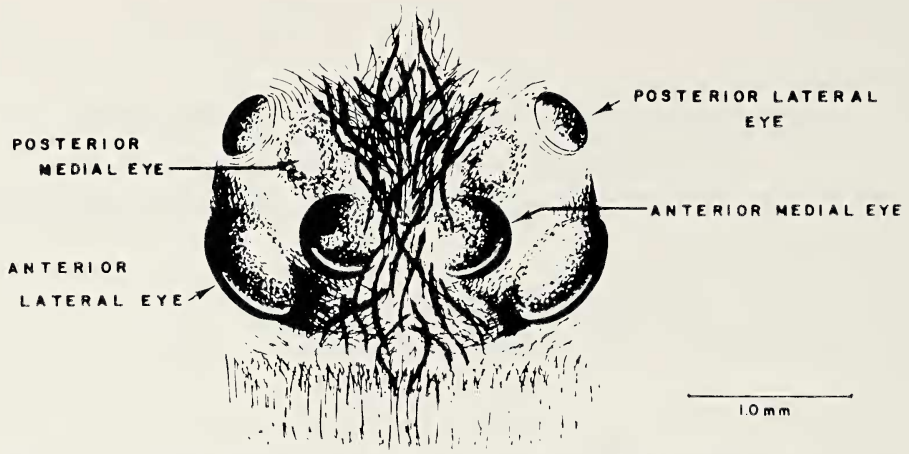

Figure 1.—Cormorus of *Aphonopelma chalcodes*. The eight eyes are arranged on the cormorus of the anterior portion of the cephalothorax as four sets of paired eyes: the *primary* Anterior Medial Eyes (AME); and the *secondary* Anterior Lateral Eyes (ALE), Posterior Medial Eyes (PME), and Posterior Lateral Eyes (PLE).

METHODS

Experimental animals.—Thirty-one female spiders were purchased from commercial sources. Their weights and pubescence were suggestive of pre-adult instars.

Preparation.—After an experimental animal was rendered tractable with carbon dioxide, the legs and the abdomen were cut off near the cephalothorax. The isolated cephalothorax was pinned dorsal surface down to a cork block in an especially constructed lucite chamber, and then immersed in spider saline solution (Rathmayer 1965). The paturons of the chelicerae and endites were removed, revealing the ocelli attached to the hemocoelic surface of the cormorus. The optic nerves were left intact. Access to the ocelli then required only minor dissection of the musculature. A 1.0 - 1.5% w/w solution of Fungal Type VI protease in saline was applied to the ocelli for one to three minutes to soften the ocellar capsule and to allow easy penetration by the electrodes.

Optical system.—A two-channel optical system was employed. One channel served for monochromatic stimulation, while the other provided light for chromatic adaptation. In Channel 1, the light from a 150W xenon arc lamp was passed through a monochromator, collimated, and focused on the tip of an ultraviolet, light-conducting, fiber-optic guide. Light intensity was controlled by neutral density wedges continuously variable over 6.65 optical density units. Light duration was controlled by an electrically operated solenoid with attached flag. An auxiliary shutter was placed in the light path of Channel 1 and used during chromatic adaptation. In Channel 2, light at either 546 nm or 365 nm from a mercury lamp was isolated by using appropriate cut-off filters; the beam was then directed onto a beam splitter in the path of Channel 1, and the combined lights were focused on the tip of the fiber optic.

The stimulus and chromatic adaptation lights were calibrated using a black-body thermopile with attached microvoltmeter. All light fluxes were specified in log photons $\cdot$ cm^{-2} $\cdot$ s^{-1} emergent from the light-guide tip.

Recording system.—Intracellular potentials were recorded from single photoreceptor cells by means of glass micropipette electrodes filled with 3M KCl. The electrodes had measured tip resistances in the range 60-80 megohms. All recordings were conventional. Permanent records were captured on film from which the measurements were made.

Experimental protocol.—Once a cell was penetrated and stable at a membrane potential between −30 mV and −40 mV, it was allowed to dark adapt for 30 min. Rapid spectral sensitivity scans, consisting of 100 ms flashes of monochromatic light over a 3 log unit range, were then made. For all spectral scans, low criterion amplitudes of 4 mV were elicited to avoid light adaptation of the preparation. Scans were done in balanced order using 370 nm and 520 nm flashes as control wavelengths. The first four flashes of each scan were control flashes followed by test flashes that in turn were interrupted by additional control flashes every fifth flash. Spectral scans spanned the spectrum from 360 nm to 640 nm, in 20 nm increments, for a total of 23 flashes per complete scan. Each scan was followed by an 11-point intensity-response series at the two control wavelengths. With interflash interval at 40 s, a complete scan took from 40 to 50 min from the time of initial impalement. Cells were also scanned under chromatic adaptation in order to determine visible and ultraviolet contributions. Such experiments were begun with a dark-adapted scan that was followed by scans under chromatic adaptation at 365 nm or 546 nm. These chromatic lights were of an intensity that elicited dark-adapted potentials of approximately 50% saturation amplitude.

RESULTS

Receptor potential.—Intracellular receptor potentials consisted of graded depolarizations to light; no regenerative responses were observed. Figure 2 shows the receptor potential of an anterior lateral eye (ALE) to monochromatic light flashes at 520 nm for three durations. These responses are typical of responses recorded from all cells. The response waveform was characterized by an initial transient depolarization that occurred to light onset, followed by a slow return of the membrane potential to its resting level. The form of the response is seen most clearly to the 100 ms flashes of Fig. 2. The transient there forms a peak which then decays slowly to the baseline from its maximum value. To longer stimulus flashes (500 and 900 ms), peak responses show longer time courses that merge with overall response decays. Even to flashes longer than 900 ms, response decays were gradual and never showed abrupt returns to baseline upon flash cessation (cf. DeVoe 1972, 1975). No differences in waveform were observed in the responses to different wavelengths of light. Occasional, irregular, transient variations in potential (*bumps*), associated with less intense lights of long duration were seen. They were similar to those reported in *Limulus* by Yeandle (1958) and probably result from membrane responses to single photon absorptions.

Peak response amplitudes varied directly as the intensity of light, although the period of depolarization was longer for longer flashes. The latency of response was inversely related to light intensity.

Intensity-response functions.—Figure 3 shows the functional relationship between response peak amplitude and light intensity. The responses were recorded to *white* light which was attenuated over a seven log unit range. The curve for the

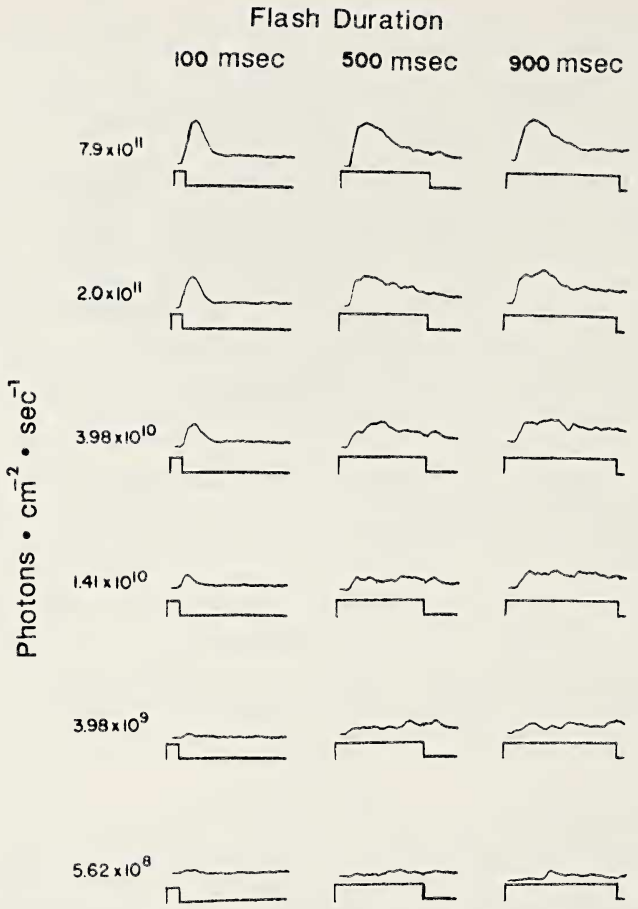

Figure 2.—Responses of ALE to monochromatic light of 520 nm. Flash durations of 100, 500, and 900 ms are listed above each column. Light intensities over a three log unit range are listed as ordinal values. An indicator for light onset and offset is shown beneath each receptor potential; its vertical displacement measures a calibrated 10.0 mV.

primary anterior median eye (AME) consists of 16 points, while the curve for a secondary anterior lateral eye (ALE) consists of 20 points. These points were fit by the Michaelis-Menten equation of format: $V/V_{max} = I^n/(I^n + k)$, where V/V_{max} is the normalized intracellular response potential, k is the intensity of light required for one-half maximum response potential, and I is an increment of light intensity (Naka and Rushton 1966; Stryer 1988:189). When comparing the two curves, the AME curve shows a greater slope coefficient than the ALE curve: the value of n is 0.71 for AME, and 0.55 for ALE. The slope differences between the two curves indicate a one log unit stimulus intensity difference at the one-half maximum response potential point in Fig. 3. This horizontal separation reflects differences in sensitivity for the two classes of eyes (Glantz 1971). The ALE cells possess an overall sensitivity advantage for dimmer lights, whereas this difference is nearly gone at about −1.5 log units where the curves begin to overlap. The linear range of sensitivity for AME is 1.5 log units, while the range for the corresponding ALE curve is 3.0 log units.

Figure 4 displays sample intensity-response functions for several sample wavelengths that plot peak amplitudes of response against light intensities for

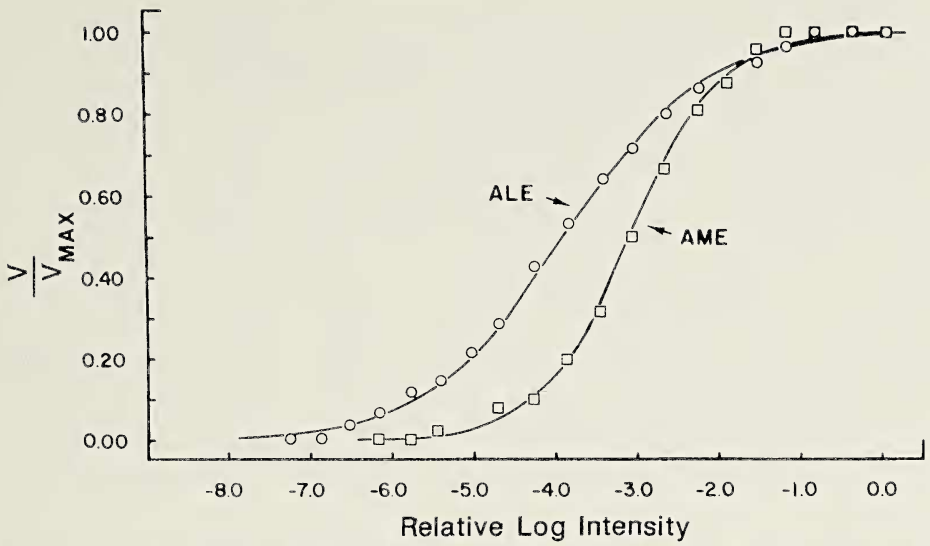

Figure 3.—Intensity-response functions to *white* light for AME and ALE cells. Amplitudes were normalized and plotted against relative log light intensity. Each point is a mean for three cells with standard deviations not larger than +/— 0.05.

AME and ALE cells. Within each kind of eye, intensity-response functions are remarkably linear, and for each eye, alike in slope regardless of stimulus wavelength. The two sets of curves differ however in their degree of slope (see Discussion).

Spectral sensitivity.—Reciprocal values of photon flux necessary to elicit criterion responses of 4.0 mV at each stimulus wavelength formed the spectral sensitivity curves. Responses of this amplitude permitted use of dim lights that did not result in appreciable light-adaptation of the cells. Action spectra for dark-adapted cells of AMEs, ALEs, PMEs, and PLEs are shown in Fig. 5. The cells of both primary and secondary eyes possess spectral sensitivity curves that are virtually identical in shape. All curves possess major sensitivity peaks near 500 nm as well as peaks of lower sensitivity in the near ultraviolet at 370 nm. Points for the longer wavelengths agree well with the Dartnall (1953) nomogram for a vitamin A_1-based photopigment peaking at 500 nm.

The measured range of wavelength sensitivity for all cells lies between 350 and 640 nm. The logarithmic difference between the sensitivity peak in the 440 nm to 640 nm range, and the sensitivity peak in the near ultraviolet, 350 nm to 400 nm, varied from 0.60 to 0.70 log unit. The overall range of sensitivity within the experimental scan, from the most sensitive point near 500 nm and the least sensitive point at 640 nm, measured 2.5 log units for all eyes.

Chromatic adaptation.—To isolate the visible or near-ultraviolet peaks, stable cells in both AME and ALE were adapted with monochromatic lights of 365 nm or 546 nm (see Methods). A representative cell is shown for each group in Figs. 6 and 7. For all light-adapted cells, overall sensitivities were reduced relative to their dark-adaptive states, but the shapes of the curves remained virtually the same. Accompanying the cells' progressive reduction in sensitivity, when adapted to 365 nm or 546 nm light, was a decrease in sensitivity difference between longer wavelength and near ultraviolet peaks. In dark-adaptation, this sensitivity

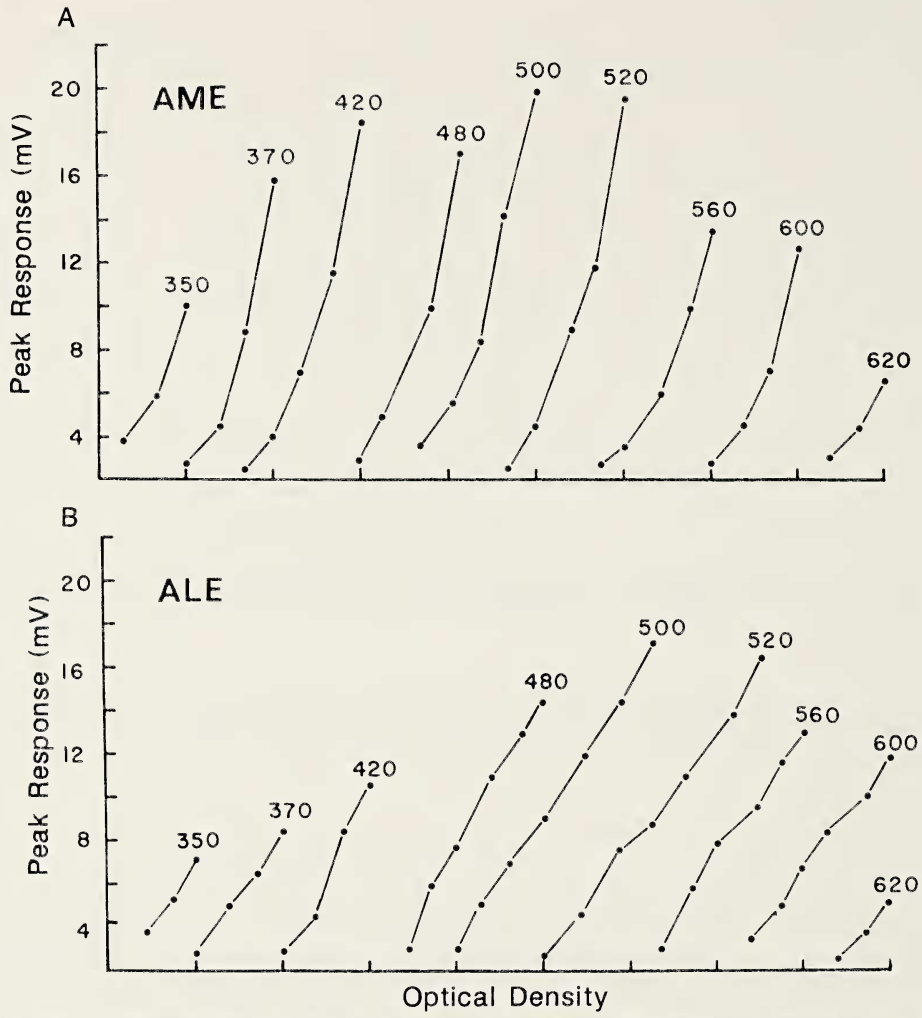

Figure 4.—A. Selected intensity-response functions for an AME cell. Response amplitude is plotted against optical density attenuation indicated as unit values on the abscissa; the response curves are separated for clarity. B. Intensity-response functions for an ALE cell is displayed as in A.

difference was 0.70 log unit, which then decreased to 0.56 log unit under chromatic adaptation to 365 nm light and to 0.35 log unit under 546 nm light.

DISCUSSION

Although primary and secondary eyes of *Aphonopelma chalcodes* display anatomical dimorphism, no functional differences were found between the two sets of eyes for two of three parameters studied. Both intracellular responses, and the spectral sensitivity functions derived from them, showed remarkable similarities. Time courses and general shapes of the waveforms did not differ between the two types of ocelli; neither did spectral sensitivity functions differ in their regions of peak sensitivity. Both primary (AME) and secondary ocelli (ALE, PME, and PLE) showed sensitivity peaks near 500 nm, with prominent peaks in

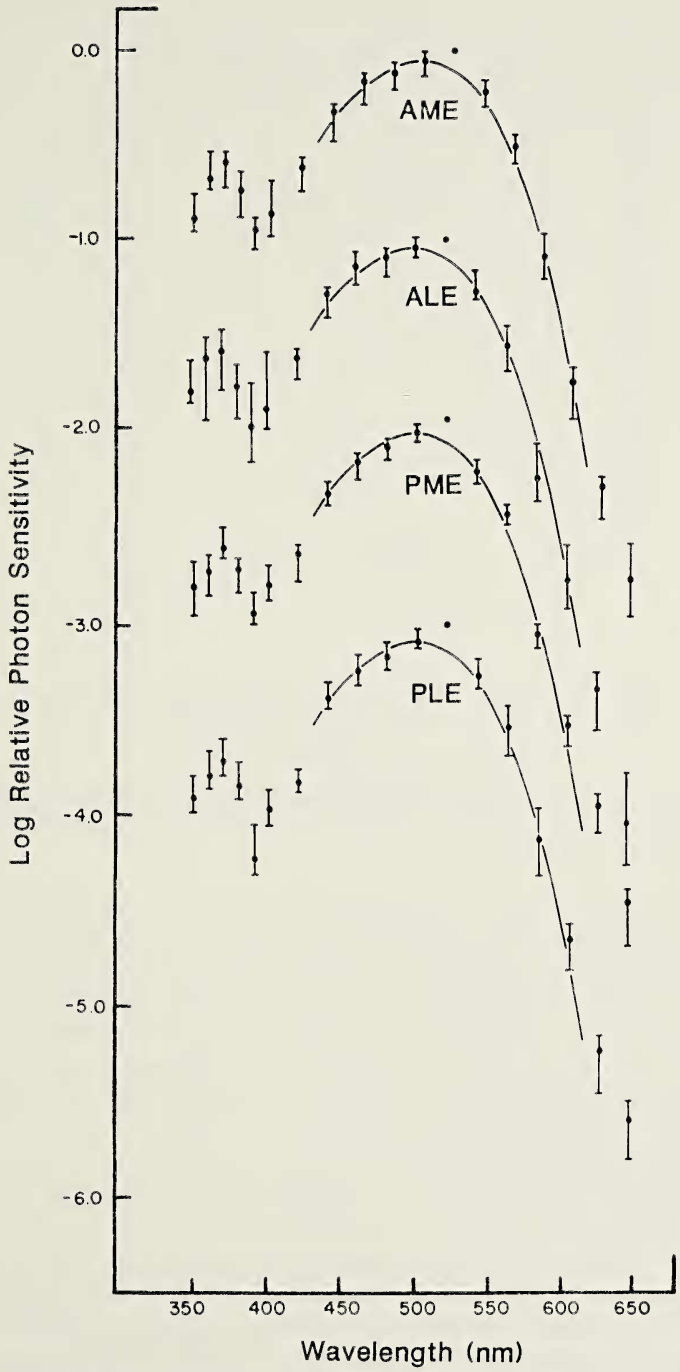

Figure 5.—Spectral sensitivity curves for AME, ALE, PME, and PLE cells. The curves are displaced vertically for clarity. Data points for each curve are mean spectral sensitivities for 12 cells normalized at 520 nm to a log relative photon sensitivity of 0.0. Vertical lines are ranges of the data points. The solid lines are derived from the nomogram of Dartnall (1953) for an A_1 photopigment absorbing maximally at 500 nm. Ordinal values related to each curve are sensitivity values derived from -log relative photon fluxes that produce 4 mV responses by 100 ms light flashes at the stimulus wavelengths in nanometers (nm) indicated on the abscissa. Mean light intensity at 500 nm was 1.2 x 10^9 photons • cm^{-2} • s^{-1}.

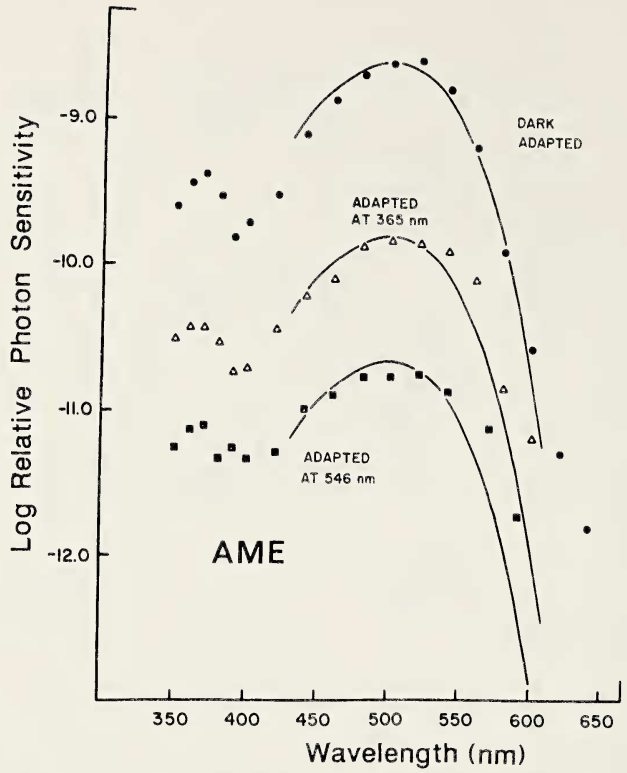

Figure 6.—Spectral sensitivity curves for a dark- and chromatically light-adapted AME cell. Solid line is derived from the Dartnall nomogram as in Fig. 5. The actual placement of individual curves derives from changes in adaptation.

the near ultraviolet at 370 nm. There was, however, a difference with respect to overall sensitivity to light. As shown in Fig. 3, the intensity-response function for the secondary ocellus ALE, showed a shallower slope coefficient (0.55) than did the function for the primary ocellus AME (0.71). The differing slopes with wavelength as a parameter can be seen in Fig. 4. Secondary ocelli are apparently able to efficiently integrate photon absorptions over a more extensive range of light intensities than do primary ocelli.

Receptor potentials and waveforms.—Receptor potentials of tarantula photoreceptors to flashes of light consist of smoothly graded depolarizations. The graded changes in membrane potential are consistent with intracellular receptor potentials recorded from other arthropod eyes and are of the same polarity (DeVoe 1975; Bruno, Mote and Goldsmith 1973). There were transient decreases in membrane potentials to light onset followed by slow restorations to initial resting levels, even with flashes longer than 100 ms. These waveforms are in contrast to those reported for wolf spider by DeVoe (1972), where prolonged flashes of light produced marked OFF responses.

Spectral sensitivity.—All photoreceptor potentials recorded from cells in AME, ALE, PLE, and PME showed a common sensitivity peak in the visible wavelengths at 500 nm, together with a lesser peak at 370 nm in the near ultraviolet, the two peaks differing in sensitivity by about 0.7 log unit. These results are in close company with the dual sensitivities that are maximum at 360-

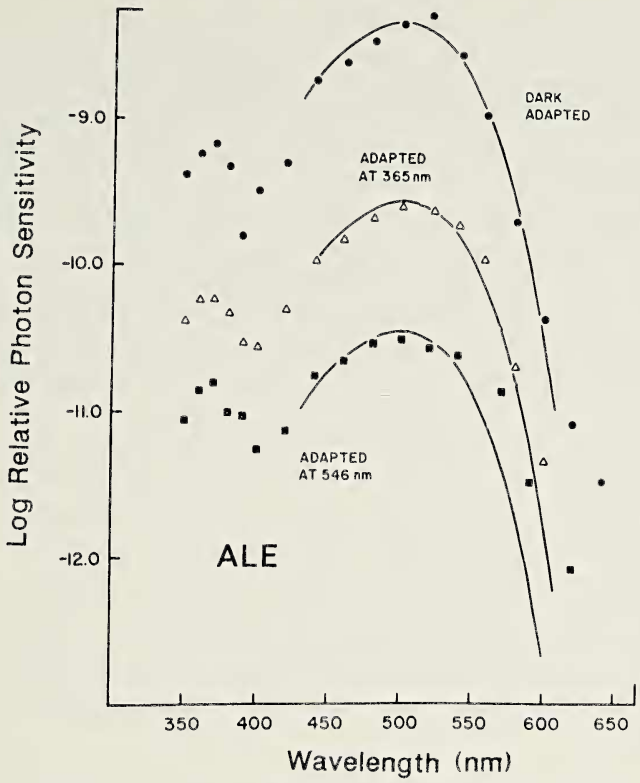

Figure 7.—Spectral sensitivity curves for a dark- and chromatically light-adapted ALE cell. See legend to Figure 6.

370 nm and at 510 nm in the principal eyes and in the anterior lateral eyes in wolf spider (DeVoe 1972). Principal eyes of the orb-weavers, *Agiope bruennichii* and *Agiope amoena*, on the other hand, have three types of receptor cells with maximum sensitivities at 360 nm, 480-500 nm, and 540 nm. Posterior lateral eyes for these same spiders show similar peak sensitivities (Yamashita 1985). In general, principal and secondary eyes of *Salticidae* and *Lycosidae*, when assessed intracellularly, have cells that correspond to *Aphonopelma* (cf. Yamashita 1985, tables 1 and 2). The excellent fit of the data points in Fig. 5 by the Dartnall nomogram supports the idea that measured spectral sensitivities most likely derive from a single vitamin A_1-based photopigment (Dartnall 1953). In further support of this idea is the fact that chromatic adaptations with 365 nm and 546 nm lights produced no differential effects. There is also evidence that parametric changes in stimulus intensity can result in matched waveforms regardless of wavelength, an argument for simple intensity effects but not one that reflects spectral changes. From all these lines of evidence, it is unlikely that *Aphonopelma* possess any ability to discriminate wavelengths.

Information over optic nerves in most animals is coded as regenerative action potentials. That is normally not the case in wolf spiders (DeVoe 1972) nor is it for *Aphonopelma* (personal observations). Photoreceptor excitation is conducted to the supraesophageal ganglion in the form of decrementing graded potentials. Although rare, nonregenerative neural activity is known to occur in other arthropod visual systems (Ionnides and Walcott 1971; Shaw 1972). The quality of

information transmitted to optic centers in this fashion must of necessity be primitive. *Aphonopelma* may therefore respond simply to the fundamental dimension of ambient light intensity. Complexities of wavelength discrimination and contour perception are apparently not involved, for the visual system here is functionally homogeneous and shows none of the response complexities seen in color discriminating eyes.

Ocellar structure and function.—Similar to many spiders, secondary ocelli in *Aphonopelma* possess tapeta. Tapeta provide a mechanism for lightpath doubling; they lie very close to the rhabdomeres so that reflected light immediately retraverses these photopigment-bearing cells (Land 1972). There are no tapeta in primary ocelli, and light traversing these rhabdomeres is absorbed by the heavily pigmented capsule of the ocellus after a single passage. When intensity-response functions for primary and secondary ocelli are superimposed, these curves reveal that at equivalent lights, and also for less intense lights, responses of secondary ocelli are larger than those of primary ocelli. This finding is consistent with the idea that secondary ocelli and associated structures have evolved as detectors of dim lights. The functions for secondary ocelli are less steep than those for primary ocelli, and this property suggests a more efficient response range for a given intensity range of light input. There appears to be in these structures a simple duplex arrangement that accounts for a greater range of light sensitivity than would otherwise be possible, an arrangement that performs for *Aphonopelma* what rods and cones do in the intensity domain for the vertebrate retina.

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